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NOTES

Metabolism of 1-Methyl-5-nitro-2-(2′-pyrimidyl)imidazole

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Abstract □ 5-Acetamido-1-methyl-2-(2′-pyrimidyl)imidazole is shown to be one metabolite of 1-methyl-5-nitro-2-(2′-pyrimidyl)-imidazole in both rats and humans. Characterization was carried out by mass spectroscopy, NMR, and IR and by synthesis of the metabolite. This first example of a metabolite reduction product of the nitro group of an imidazole is discussed in relation to previous studies.

Keyphrases □ 1-Methyl-5-nitro-2-(2′-pyrimidyl)imidazole—metabolism, identification of 5-acetamido-1-methyl-2-(2′-pyrimidyl)imidazole as metabolite □ 5-Acetamido-1-methyl-2-(2′-pyrimidyl)imidazole—identified as metabolite of 1-methyl-5-nitro-2-(2′-pyrimidyl)imidazole □ Metabolism—1-methyl-5-nitro-2-(2′-pyrimidyl)imidazole

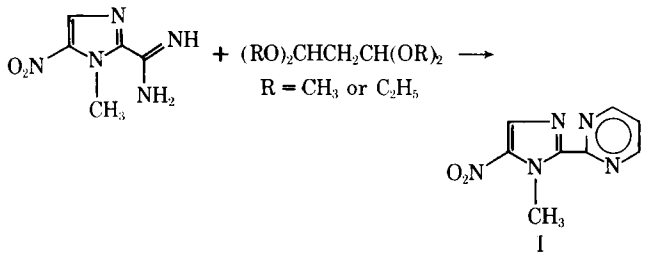
The drug 1-methyl-5-nitro-2-(2′-pyrimidyl)imidazole (I) was recently synthesized according to Scheme I (1). The drug I shows antitrichomonal properties both *in vitro* and *in vivo* against *Trichomonas vaginalis* in mice. During studies on the absorption, excretion, and metabolism of the drug, one major metabolite of I was noticed in the urine of both rats and humans. This paper presents data on the isolation, identification, and synthesis of this metabolite and shows it to be a product of reduction of the nitro group, with the structure 5-acetamido-1-methyl-2-(2′-pyrimidyl)imidazole.

Previous work on the metabolism of the nitroimidazole class of drugs (2, 3) showed that amines may be formed as metabolic reduction products of the nitro group, but they have never been isolated due to the extreme instability of 5-aminoimidazole derivatives. Work on the nitrofurfuraldehyde derivative class of drugs has shown (4, 5) that acetylated amine derivatives can be found as metabolites, and the first example of such a metabolite from the 5-nitroimidazole series is presented here.

EXPERIMENTAL[1]

1-Methyl-5-nitro-2-(2′-pyrimidyl)imidazole (I)—This com-

[1] Melting points were determined with a Büchi capillary apparatus and are uncorrected. IR spectra were obtained on a Hilger-Watts spectrophotometer, and UV spectra were obtained on a Beckman DB-GT spectrophotometer with a Sargent-Welch SRG recorder. NMR spectra were determined with a Perkin-Elmer R12-B spectrometer using tetramethylsilane as the internal reference. Mass spectra were obtained on an LKB 9000 instrument equipped with a gas chromatograph and a 3% SE-30 column (2 m long), operating at 250°. The ion beam energy was 70 ev, the ion source temperature was 290°, the accelerating voltage was 3.5 kv, and the trap current was 60 μamp. GLC was carried out on a Carlo Erba Fractovap GI chromatograph equipped with a flame-ionization detector using a 3% SE-30 glass column (2 m long). The operating conditions were: injection port temperature, 270°; oven temperature, 250°; nitrogen (carrier gas) flow rate, 30 ml/min; hydrogen flow rate, 100 ml/min; and air flow rate, 280 ml/min. TLC was carried out on Merck F_{254} silica gel plates, and column chromatography was carried out on Florisil, 100–200 mesh (British Drug Houses).



Scheme I

pound was prepared according to published procedures (1) and showed the following characteristics: mp 205–207°; UV (ethanol): λ_{max} 314 nm, $E_{1cm}^{1\%}$ 655; IR (KBr): 1570 and 1537 cm^{-1}; NMR ($CDCl_3$): δ 4.42 (s, 3, N—CH_3), 7.40 (t, 1, C-5′ pyrimidyl H), 8.12 (s, 1, imidazole H), and 8.93 (d, 2, C-4′ and C-6′ pyrimidyl H); mass spectrum: M^+ 205.

5-Acetamido-1-methyl-2-(2′-pyrimidyl)imidazole (II)—Compound I (4.1 g, 0.02 mole) in acetic acid (20 ml) was treated with zinc powder (10 g) for 10 min at 15°, and the solution was filtered and treated with 20 ml of acetic anhydride for 15 min at 60°. After the solution was brought to dryness, the residue was dissolved in 50 ml of water at 20° and successively treated with hydrogen sulfide until the complete precipitation of zinc sulfide. Then the solution was filtered and dried, and the residue was dissolved in 10 ml of ethanol and chromatographed on a 100–200-mesh magnesium silicate[2] column (20 cm high, 3 cm i.d.). The first eluant added was 350 ml of ethanol–acetone (1:1), and later 400 ml of ethanol–acetone–water (2:2:1) was added. The solution was dried and recrystallized from ethanol, yielding 160 mg II, mp 224–228°; UV (ethanol): λ_{max} 295 nm, $E_{1cm}^{1\%}$ 715; IR (KBr): 3240, 3220, and 1710 cm^{-1}; NMR ($CDCl_3$): δ 2.20 (s, 3, $COCH_3$), 3.90 (s, 3, N—CH_3), 7.10 (t, 1, C-5′ pyrimidyl H), 7.26 (s, 1, imidazole H), and 8.70 (d, 2, C-4′ and C-6′ pyrimidyl H); mass spectrum: M^+ 217.

Anal.—Calc. for $C_{10}H_{11}N_5O$: C, 55.28; H, 5.10; N, 32.25. Found: C, 54.98; H, 5.05; N, 32.26.

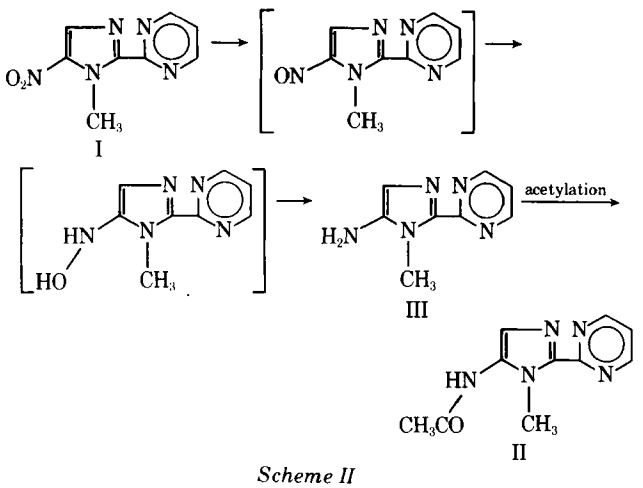
Animal Studies—Compound I was administered orally every 24 hr for 5 days (300 mg/kg/day) as a suspension in carboxymethylcellulose (0.375%) to male Wistar rats weighing 100–120 g. The animals were fasted for 24 hr prior to the experiments, but food was administered regularly during the experiments. After drug administration, the urine was collected for 5 days from both the treated group and a parallel control group of untreated rats.

TLC and GLC Studies—Urine (50 μl), concentrated 10:1 with a rotary evaporator[3] at a temperature not exceeding 50° from each treated and untreated animal, was examined on silica plates together with authentic samples of I and II. Plates were developed to 15 cm using the following solvent systems: A, *n*-propanol–ammonia (7:3); and B, acetone–ethanol–water (2:2:1).

After development, UV chromophores were located with UV light of 254 and 360 nm, and there were two major spots corresponding to the metabolite II and the unchanged drug I. The R_f values of I and II in Solvent System A were 0.94 and 0.90, respectively; in Solvent System B, they were 0.21 and 0.90, respectively.

Urine (10 ml) at pH 10 was extracted twice with 20 ml of chloroform, filtered through anhydrous sodium sulfate, and evaporated to dryness at reduced pressure. The extracts were dissolved in 100 μl chloroform–ethanol (1:1), and 3-μl samples were injected in a 5% SE-30 column. The samples were chromatographed isothermally at 200° for 6 min, programmed (10°/min) from 200 to 250°, and kept isothermally at 250° for 5 min. Two major peaks were obtained with retention times identical to those of I and II.

Isolation of I and II—Urine (400 ml) was adjusted to pH 10–11 with 0.1 *N* NaOH and extracted with chloroform as described previously. The residue was dissolved in chloroform–ethanol (1:1) and placed on a column of magnesium silicate, 100–200 mesh (2 × 15 cm). The column was washed with chloroform (100 ml), and the chloroform washings were discarded. Then the column was eluted with acetone–ethanol (1:1) to give Fraction A (150 ml) and with acetone–ethanol–water (2:2:1) to give Fraction B (150 ml).



Scheme II

Examination by TLC using the described solvent systems showed that Fraction A contained mostly unmetabolized I while Fraction B contained mostly the major metabolite II.

Both fractions were rechromatographed in the same way. The first eluate from Fraction A contained pure I while the pure metabolite II was obtained in the acetone–ethanol–water (2:2:1) eluate from the rechromatography of Fraction B.

Fraction A—The material from Fraction A was recrystallized from acetone–petroleum ether to give material, mp 205–206°, identical with Compound I.

Fraction B—The material from Fraction B was dissolved in 1 *M* hydrochloric acid. The acid solution was washed twice with chloroform that was then discarded. Then the solution was made basic with 1 *M* NaOH and extracted with chloroform, the chloroform was brought to dryness, and the residue was treated with ethanol and filtered. The filtrate was concentrated and recrystallized twice from ethanol to give material, mp 224–228°, identical to that of Compound II. The UV, IR, NMR, and mass spectra of the extracted metabolite were identical to those of synthetic II.

RESULTS AND DISCUSSION

1-Methyl-5-nitro-2-(2′-pyrimidyl)imidazole (I) was administered to rats as already described, and the urine contained only one major metabolite [R_f 0.21, acetone–ethanol–water (2:2:1)] not present in the urine of untreated rats. This metabolite was also present in the urine of human subjects[4]. The unchanged starting material and the metabolite were separated as described, and each was identified by spectral means and comparison with authentic samples.

The metabolite having R_f 0.21 in acetone–ethanol–water (2:2:1) had IR absorption bands at 3240 and 3220 cm^{-1}, indicating an amine or an amide, and 1710 cm^{-1}, indicating a carbonyl group. The bands at 1570 and 1537 cm^{-1} had disappeared, indicating that metabolic attack had occurred at the nitro group. The bands present were consistent with the presence of an acetamido group in the molecule. NMR spectroscopy showed that, in comparison to I, the compound had acquired a singlet methyl resonance at δ 2.20 attributable to an acetyl group, the *N*-methyl resonance had shifted from δ 4.42 to 3.91, and the C-4 proton of the imidazole ring had shifted from δ 8.12 to 7.26.

These data were consistent with the conversion of the nitro group to an acetamido group. The mass spectrum of the metabolite (II) was obtained using GLC–mass spectroscopy and showed (Fig. 1) a molecular ion at *m/e* 217 and a primary loss of 42 amu (CH_2=C=O) with a metastable ion to give the base peak at *m/e* 175. The loss of 42 amu confirms the presence of an acetyl group. The molecular ion at *m/e* 217 is consistent with a molecular formula $C_{10}H_{11}N_5O$ and with the structure 5-acetamido-1-methyl-2-(2′-pyrimidyl)imidazole. The mass spectrum of the drug I (Fig. 1) shows a molecular ion at *m/e* 205 and fragment ions at *m/e* 189 (M − O) and 175 (M − NO).

[2] Florisil.
[3] Büchi rotavapor.

[4] G. Sekules and M. Coerezza, unpublished results.

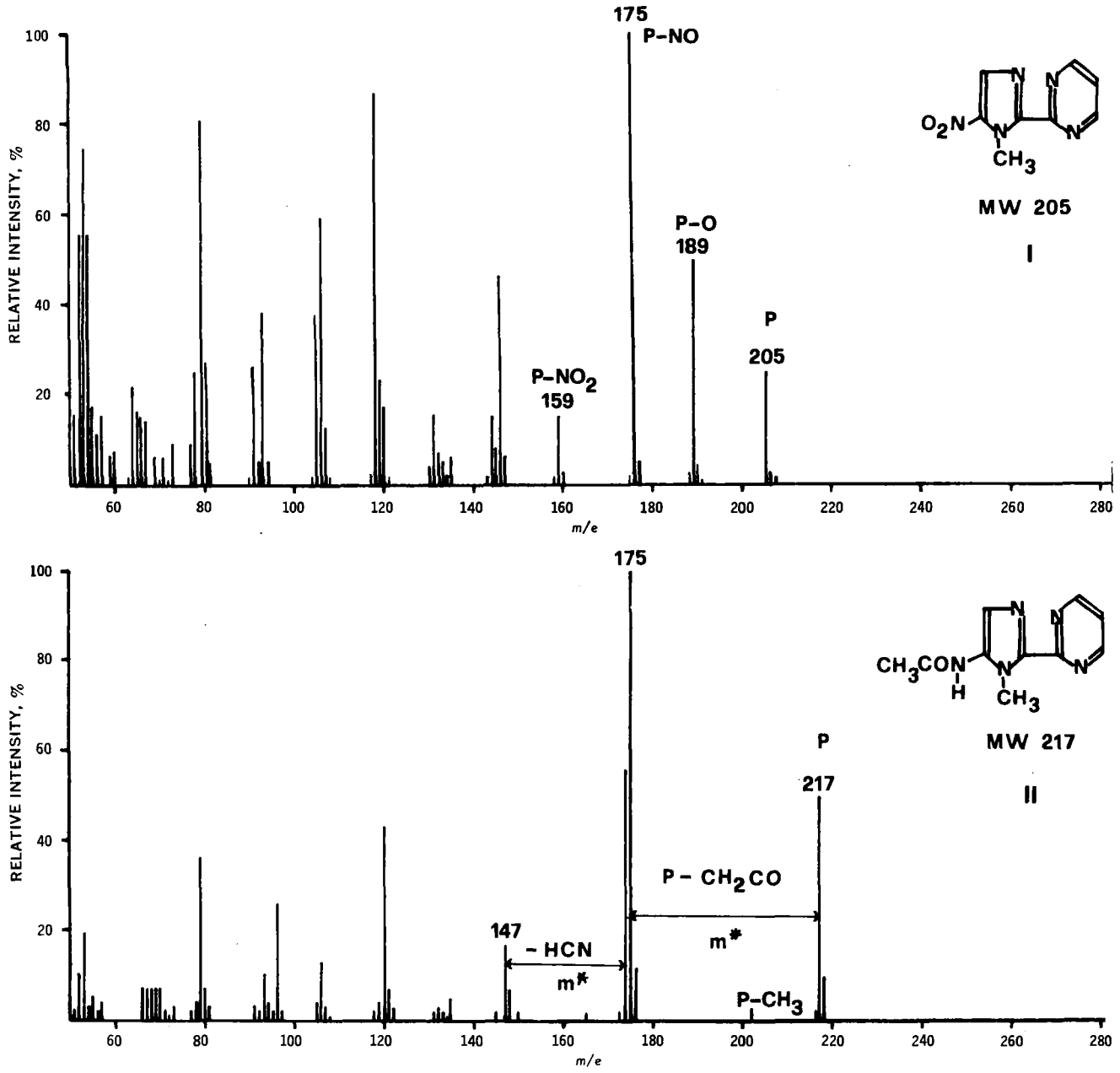


Figure 1—*Mass spectra of drug 1-methyl-5-nitro-2-(2'-pyrimidyl)imidazole (I) (upper) and metabolite 5-acetamido-1-methyl-2-(2'-pyrimidyl)imidazole (II) (lower) obtained by GLC–mass spectroscopy.*

To confirm the structure assignment, the acetamide was synthesized as already described. Reduction of the nitro group to form the amine (III) proved difficult; and once formed the amine was unstable, giving rise to a number of products. Acetylation of the amine gave the required material, II, which was identical in all its physical properties with the metabolite isolated from urine. The synthesized II also contained very minor amounts of impurities, which probably resulted from the decomposition of the amine intermediate in the synthesis. These minor impurities were also present in the urine of the treated rats, and hence it seems likely that the amine (III) is an intermediate in the metabolic pathway. A proposed scheme for the metabolic pathway is shown in Scheme II.

The *in vivo* acetylation of the amine forms a stable derivative II which allows its isolation. This is the first example of the isolation of a metabolite resulting from the reduction of the nitro group in the nitroimidazole class of drugs, and the inability to isolate amines previously was doubtless due to the extreme instability of that class of compound (3).

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